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Composition and bioactivity assessment of essential oils of *Curcuma longa* L. collected in China

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A R T I C L E I N F O

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A B S T R A C T

Curcuma longa L. rhizomes collected from 20 different habitats in China are used to analyze yield, composition and bioactivity of essential oils extracted from them. The yield of the 20 essential oils vary from 4.03 to 5.27% depending on the habitat where they were collected. Using gas chromatography–mass spectrometry (GC–MS), 81 components are identified in the 20 essential oils, and their major compounds are *ar*-turmerone (0.92–42.85%), β-turmerone (5.13–42.54%), α-zingiberene (0.25–25.05%), *ar*-curcumene (1.21–15.70%) and β-sesquiphellandrene (0.05–14.88%). The essential oils of rhizomes collected from different habitats exhibit different antimicrobial activities, and the essential oils from Guangxi have generally better activities. These essential oils also show different DPPH (IC_{50}, 4.37–11.59 μg/mL) and ABTS (IC_{50}, 4.21–13.25 μg/mL) radical-scavenging activities, being most of them more effective than Trolox C (IC_{50}, 10.72 and 11.42 μg/mL). They also exhibit significantly different cytotoxicity against B16 cells (IC_{50}, 13.96–135.97 μg/mL) and LNCaP cell (IC_{50}, 19.63–127.81 μg/mL), and essential oils from Luchuan in Guangxi have the highest cytotoxicity. Some essential oils show outstanding anti-inflammatory activities by markedly down-regulating the expression of inflammatory cytokines, cyclooxygenase 2 (COX-2) and tumor necrosis factor (TNF)-α, *in vivo*. Therefore, *Curcuma longa* L. rhizome, one of the popular traditional Chinese medicines with excellent bioactivities, can be more rationally utilized based on their chemical composition and bioactivity in the further.

1. Introduction

Of more than 110 species of genus *Curcuma* in the family Zingiberaceae, only about 20 species have been investigated phytochemically (Li et al., 2011). *Curcuma longa* L. is one of the most investigated *Curcuma* species, due to its rhizome is widely used as dye, flavoring agent and Chinese herb. *C. longa* is distributed throughout tropical and subtropical regions in the world, being widely cultivated in Asiatic countries, especially in India and China. This species, called Jianghuang or Huangjiang in China, has been used in cosmetic, food and medicine. As one of the popular Chinese herbs, *C. longa* rhizome is often used to treat gastric ulcers, parasitic infections, skin disorders, sprains, joint inflammation and cold and flu symptoms (Harsha et al., 2016; Sahne et al., 2016). In generally, the bioactivities of *C. longa* rhizome are mainly anti-inflammatory, antioxidant, antimicrobial, anticancer and anti-viral (Chaithra et al., 2016).

To date, at least 235 compounds have been identified in *C. longa*, such as an oleoresin consisting of a yellowish-brown heavy fraction containing curcuminoids and a light fraction containing essential oils. The chemical compounds of essential oils extracted *C. longa* rhizome belong to monoterpenes and sesquiterpenes, particularly zingiberene, *ar*-turmerone, α-turmerone, β-turmerone and germacrone, which vary with geographic location of the harvested plant (Hossain and Ishimine, 2005). Fifty-four compounds in essential oils of *C. longa* (yellow type) have been identified, in which the major compounds are *ar*-turmerone (27.78%) and turmerone (17.16%). Only 39 compounds have been identified in *C. longa* (red type), with carvacrol (21.14%) and citral (13.91%) as the major constituents (Chowdhury et al., 2008). In addition, the essential oils of *C. longa* collected from Jiangxi, Fujian and Sichuan provinces are analyzed, and β-turmerone (17.27–31.43 mg/g), α-turmerone (14.58–21.87 mg/g) and *ar*-turmerone (7.55–12.63 mg/g) are determined to be the major components (Avanço et al., 2017; Qin et al., 2007).

One major problem with the medical usage of *C. longa* rhizome

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collected in China is the high degree of variation in the phytoconstituent concentrations, which is dependent on agroclimatic conditions (Anandaraj et al., 2014; Zhang et al., 2017a). The yield and quality of essential oils, also vary with genetics, agroclimatic conditions, planting technique, soil conditions, harvest time, etc. Therefore, chemical compositions of essential oils of *C. longa* rhizome vary in different habitats, and their bioactivity is closely associated with compositions will change, spontaneously. In China, *C. longa* is mainly distributed over Guangxi, Sichuan, Guangdong and Yunnan provinces. However, the chemical compositions and bioactivity of essential oils of *C. longa* rhizome collected from different habitats in China, have not been comprehensively studied. In view of the importance of *C. longa* as famous Chinese herb, chemical compositions of essential oils of *C. longa* rhizome collected from different habitats in China are to be characterized by GC–MS in this study, and their bioactivity including antioxidant, antimicrobial, anti-inflammatory and anti-tumor and activities will be investigated.

2. Materials and methods

2.1. Plant materials

The *C. longa* rhizomes used in this study were collected from 20 different habitats in Guangxi, Sichuan, Chongqing, Yunnan and Guangdong provinces. The specific geographic information of the habitats is shown in Table 1. Each habitat was given a specific accession CLX (X = 1–20) for the convenient discussion. The rhizomes of *C. longa* were used to cultivate, and the plants were grown under natural conditions of photoperiod and temperature. After about ten month, matured rhizomes were harvested in January and February, because at this period the content and quality of essential oil were richest (Chen and Zeng, 2008). All plant materials were identified by Prof. Nian Liu (Zhongkai University of Agriculture and Engineering, Guangzhou, China) according to the morphological description presented in The Zingiberaceae Resource of China (Wu, 2015).

The fresh rhizomes were manually cleaned with water to remove adhering soil and extraneous matter. Then, rhizomes were sliced, air-dried under room temperature (~25 °C), and grinded into powder. The powder (50 g) was subjected to hydro-distillation for 3.5 h using a Clevenger-type apparatus. The distilled essential oils were then dried over anhydrous $MgSO_4$ and preserved in dark tubes at 4 °C until use. The essential oil yield was calculated according to the following formula:

Essential oil yield (%) = obtained essential oil (g)/dried rhizome - sample (g) × 100

2.2. Gas chromatography–mass spectrometry (GC–MS) analysis

Approximately 1 μL of essential oil was analyzed using a DSQ-II Ultra GC–MS (Thermo, USA) with helium as the carrier gas at a flow rate of 1.0 mL/min. The GC–MS was equipped with a 30 m long DB-5MS capillary column with 0.25-μm film thickness (Agilent, USA), and a split ratio of 100:1. The temperature program was used as follows: column was maintained at 40 °C for 1 min and raised 3 °C/min to 280 °C, where it was maintained for 5 min. The instrument was set with an electron energy of 70 eV, ion source temperature of 230 °C, and was used in electron-impact mode. The components were identified by comparing their recorded mass spectra with General Purpose, Terpene ThermoQuest, NIST libraries and literatures (Adams, 2007; Babushok et al., 2011; Yang et al., 2011).

2.3. Minimum inhibitory concentrations (MIC)

Escherichia coli (ATCC25922), *Pseudomonas aeruginosa* (ATCC15442), *Staphylococcus aureus* (ATCC6538), *Candida albicans* (ATCC10231) and *Saccharomyces cerevisiae* (GIM-2) were donated by Guangdong Institute of Microbiology (Guangzhou, China). The minimum inhibitory concentration (MIC) of essential oils were determined by a broth microdilution method. The broth cultures were added to a 96-well plate, and the final concentrations of bacterial were adjusted to 5.0×10^5 CFU/mL after 24 h cultivation. Then, various concentrations of essential oils were added to the suspensions. After incubating at 37 °C for 24 h, the number of surviving organisms was calculated by viable counts. When the viability was about 90%, the lowest concentration was selected as the MIC of that essential oil. Each experiment was performed in triplicate (Cosentino et al., 1999).

2.4. Antioxidant activity

The antioxidant activity of the 20 essential oils were firstly estimated by 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay. Various concentrations of essential oils (200 μL) were mixed with 3.0 mL of 5.25×10^{-5} mol/L DPPH in absolute ethanol, respectively. The absorbance at 517 nm of tested mixtures was monitored after 30 min with a Perkin-Elmer Lambda 25 UV/V spectrophotometer. Trolox C was used as a positive control. The DPPH radical-scavenging activities (*RSA%*) of

Table 1
Geographic and climatic information on the collected *C. longa* rhizomes for the study.

Province	District	Locality	Longitude	Latitude	Accessions collected	Climate	Elevation
Guangxi	Guigang	Gangnan	109°35′	23°04′	CL1	subtropical monsoon climate	47.71m
	Yulin	Yuzhou	110°08′	22°37′	CL2	subtropical monsoon climate	78.08m
	Yulin	Luchuan	110°15	22°19′	CL3	subtropical monsoon climate	100.60m
	Nanning	Xingning	115°43′	24°08′	CL4	subtropical monsoon climate	121.85m
	Nanning	Hengxian	109°15′	22°40′	CL5	subtropical monsoon climate	59.79m
	Qinzhou	Lingshan	109°17′	22°25′	CL6	subtropical monsoon climate	62.91m
Sichuan	Chengdu	Shuangliu	103°55′	30°34	CL7	Subtropical monsoon humid climate	498.32m
	Leshan	Muchuan	103°54′	28°57	CL8	subtropical monsoon climate	398.89m
	Leshan	Jianwei	103°56′	29°12′	CL9	subtropical monsoon climate	340.06m
	Luzhou	Luxian	105°22′	29°09′	CL10	subtropical humid monsoon climate	285.50m
	Yibin	Jiang'an	105°03′	28°43	CL11	subtropical humid monsoon climate	274.93m
	Yibin	Shiqu	104°38′	28°45′	CL12	subtropical monsoon climate	321.27m
	Panzhihua	Yanbian	101°51′	26°41′	CL13	Subtropical dry valley climate	1143.23m
	Suining	Shehong	105°23′	30°52′	CL14	subtropical monsoon climate	337.73m
	Chengdu	Wenjiang	103°51′	30°41′	CL15	subtropical humid monsoon climate	528.19m
Chongqing	Hechuan	Xianglong	106°34′	30°15′	CL16	subtropical monsoon climate	301.10m
Yunnan	Yuxi	Tonghai	102°45′	24°06′	CL17	subtropical monsoon climate	1831.19m
	Wenshan	Maguan	104°23′	23°0′	CL18	subtropical monsoon climate	1320.80m
Guangdong	Zhaoqing	Gaoyao	112°27′	23°01′	CL19	subtropical monsoon climate	34.43m
	Guangzhou	Panyu	113°22′	22°56′	CL20	subtropical monsoon climate	2.71m